

Preclinical efficacy and safety of mepolizumab (SB-240563), a humanized monoclonal antibody to IL-5, in cynomolgus monkeys

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Background: Allergic respiratory diseases are characterized by large numbers of eosinophils and their reactive products in airways and blood; these are believed to be involved in progressive airway damage and remodeling. IL-5 is the principal cytokine for eosinophil maturation, differentiation, and survival. Mepolizumab (SB-240563), a humanized monoclonal antibody (mAb) specific for human IL-5, is currently in clinical trials for treatment of asthma.

Objective: The purpose of this study was to characterize the pharmacologic activity and long-term safety profile of an anti-human IL-5 mAb to support clinical trials in asthmatic patients.

Methods: Naive and *Ascaris suum*-sensitive cynomolgus monkeys received various dose levels of mepolizumab and were monitored for acute and chronic pharmacologic and toxic responses.

Results: To support preclinical safety assessment, cynomolgus monkey IL-5 was cloned, expressed, and characterized. Although monkey IL-5 differs from human IL-5 by 2 amino acids (Ala27Gly and Asn40His), mepolizumab has comparable inhibitory activity against both monkey IL-5 and human IL-5. In *A suum*-sensitive monkeys, single doses of mepolizumab significantly reduced blood eosinophilia, eosinophil migration into lung airways, and levels of RANTES and IL-6 in lungs for 6 weeks. However, mepolizumab did not affect acute bronchoconstrictive responses to inhaled *A suum*. In an IL-2-induced eosinophilia model (up to 50% blood eosinophilia), 0.5 mg/kg mepolizumab blocked eosinophilia by >80%. Single-dose and chronic (6 monthly doses) intravenous and subcutaneous toxicity studies in naive monkeys found no target organ toxicity or immunotoxicity up to 300 mg/kg. Monkeys did not generate anti-human IgG antibodies. Monthly mepolizumab doses greater than 5 mg/kg caused an 80% to 100% decrease in blood and bronchoalveolar lavage eosinophils lasting 2

months after dosing, and there was no effect on eosinophil precursors in bone marrow after 6 months of treatment.

Eosinophil decreases correlated with mepolizumab plasma concentrations (half-life = 13 days).

Conclusion: These studies demonstrate that chronic antagonism of IL-5 by mepolizumab in monkeys is safe and has the potential, through long-term reductions in circulating and tissue-resident eosinophils, to be beneficial therapy for chronic inflammatory respiratory diseases. (*J Allergy Clin Immunol* 2001;108:250-7.)

Key words: IL-5, monoclonal antibody, asthma, eosinophilia, monkey, preclinical safety, toxicology

Asthma is a complex disorder characterized by eosinophilic inflammatory infiltrates and intermittent, reversible airway obstruction and hyperresponsiveness. In response to allergen, allergic asthmatics exhibit a biphasic bronchoconstrictor response with early- and late-phase reactions. The early response is initiated by bronchospastic mediator release. The late phase is characterized by the presence of CD4-positive T cells that express mRNA for the T_H2-type cytokines, IL-4 and IL-5, with an associated eosinophilia and pulmonary inflammation. There is increasing evidence to suggest that eosinophils play a central role in asthma pathogenesis through release of proinflammatory and cytotoxic molecules; these molecules are believed to be the major cause of smooth muscle hypertrophy and chronic mucosal damage, leading to airway hyperreactivity and deterioration of lung function over time.¹⁻³

IL-5⁴ is the major hematopoietin responsible for growth, differentiation,⁵ recruitment,⁶ activation, and survival⁷ of eosinophils and is therefore considered an important factor in asthma pathogenesis⁸ and other diseases.⁹ Administration of anti-IL-5 mAbs prevents eosinophil infiltration into airways and development of bronchial hyperresponsiveness in mouse¹⁰ and primate¹¹ models of allergic asthma. In addition, anti-IL-5 mAbs prevent antigen-¹² or leukotriene-induced¹³ eosinophil infiltration and bronchial hyperreactivity in guinea pig airways. Thus, animal models show anti-IL-5 mAbs to be effective in attenuating the late-phase response and are believed to be a good model of clinical asthma. These observations suggest that eosinophil levels and IL-5 play

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Abbreviations used

BAL:	Bronchoalveolar lavage
C _{DYN} :	Dynamic compliance
HPF:	High-power field
iv:	Intravenous (intravenously)
R _L :	Lung resistance
sc:	Subcutaneous (subcutaneously)

a prominent role in maintaining the chronic inflammatory state in airways of individuals with asthma. In recent clinical studies, the anti-IL-5 mAb SB-240563 and IL-12, while reducing the number of circulating eosinophils, did not affect early- or late-phase responses in subjects with asthma.^{14,15} However, these were acute treatment studies, and chronic therapy might be required to reduce tissue eosinophil levels to affect these endpoints.

Mepolizumab (SB-240563) is a fully humanized mAb specific for human IL-5. Mepolizumab blocks binding of human IL-5 to the α chain of the IL-5 receptor complex expressed on the cell surface of eosinophils. It is predicted that mepolizumab will reduce the accumulation of eosinophils in asthmatic lungs, reduce activation of infiltrated cells, and reduce elevated levels of circulating eosinophils. Mepolizumab might also have the potential to reverse airway remodeling, which in severe cases leads to permanent tissue destruction. Before evaluation of mepolizumab in human beings, preclinical pharmacology, safety, and toxicity studies were conducted in cynomolgus monkeys, the only animal model in which the antibody displays cross-reactivity. The clinical development of mepolizumab in asthma has begun with single-dose phase I studies¹⁴ and is currently in repeat-dose phase II studies.

MATERIALS AND METHODS

Mepolizumab was constructed through use of conventional molecular techniques to graft complementarity-determining regions from a parent murine mAb 2B6,¹⁶ raised against recombinant human (rh) IL-5, into human IgG₁ (κ) heavy and light chains. Additional amino acid substitutions were made to reflect human immunoglobulin group/subgroup preferences. Mepolizumab's binding affinity (K_d) for rhIL-5 is 4.2 pM. A stable Chinese hamster ovary cell line producing multigram quantities and a purification procedure were identified. Supplies were prepared as sterile solutions or lyophilic products at GlaxoSmithKline Pharmaceuticals (King of Prussia, Pa).

Study designs

Single-dose pharmacology study. Eight male cynomolgus monkeys were selected on the basis of positive bronchoconstrictor responses to inhaled *Ascaris suum* antigen. Monkeys received 10 mg/kg mepolizumab or vehicle (4 per group) by intravenous (iv) injection. Each animal's untreated response to antigen was evaluated 2 weeks before drug administration; an aerosol challenge was performed with 15 breaths of *A suum* antigen in PBS via an ultrasonic nebulizer. At 24 hours and at 3 and 6 weeks after dosing, monkeys received aerosol challenges of *A suum*. Monkeys were anesthetized, intubated, and maintained on a ventilator for each procedure. The following were evaluated before and 24 hours after

each antigen challenge: hematology; pulmonary function (lung resistance [R_L] and dynamic compliance [C_{DYN}]); bronchoalveolar lavage (BAL) absolute and differential cell counts; and RANTES, IL-6, and IL-8 concentrations.

Single-dose toxicity and pharmacology studies. Mepolizumab was administered iv at dosages of 0, 3, or 300 mg/kg to monkeys (1/sex/group). Each animal was monitored for 1 month and then killed; a complete necropsy was performed. The monkeys were evaluated for all of the following: clinical observations, body weight, hematology, hemostasis, serum chemistry, urinalysis, leukocyte phenotyping, mitogen responses, anti-mepolizumab antibodies, toxicokinetics, organ weights, and histology.

Male cynomolgus monkeys (n = 3), implanted with femoral artery vascular access ports were given vehicle (2 times) and 10 and 100 mg/kg mepolizumab iv at weekly intervals. The following were monitored for 3 hours after dosing: clinical observations, body weight, blood pressures, heart rate, urinary excretion (osmolality, sodium, potassium, chloride, and creatinine), serum electrolytes (sodium, potassium, and chloride), serum creatinine, respiratory rate, and body temperature.

Two-dose toxicity and pharmacology study. Mepolizumab was administered iv at a dosage of 0, 0.05, 0.5, 5, or 50 mg/kg to monkeys (2/sex/group) on days 1 and 29. Monkeys received 6 subcutaneous (sc) doses (1 dose every other day) beginning on day 30 of Proleukin (rhIL-2; 22 μ g/kg/dose; Chiron Therapeutics, Emeryville, Calif) to induce a peripheral blood eosinophilia. Monkeys were monitored for 17 weeks. They were evaluated for all of the following: clinical observations, body weight, hematology, hemostasis, serum chemistry, urinalysis, leukocyte phenotyping, anti-mepolizumab antibodies, toxicokinetics, electrocardiography, and ophthalmology.

Six-month toxicity study. Mepolizumab was administered iv (0, 10, or 100 mg/kg) or sc (0 or 10 mg/kg) once per month for 6 months to monkeys (3/sex/group). These were the same monkeys used in the 2-dose toxicity and pharmacology study. Monkeys were killed 1 week after the last dose. They were evaluated for all of the following: clinical observations, body weight, hematology, hemostasis, serum chemistry, urinalysis, lymphocyte phenotyping, anti-mepolizumab antibodies, toxicokinetics, electrocardiography, ophthalmology, BAL (total and differential cell counts and protein levels), organ weights, and histology.

All study designs were reviewed and approved by institutional Animal Care and Use Committees. Definitive toxicity studies were conducted in accord with Good Laboratory Practices for Nonclinical Laboratory Studies (21 CFR, part 58).

Procedures

Cloning and expression of cynomolgus monkey IL-5. Poly-A mRNA from spleen (1 g) of a female monkey and a male monkey was isolated through use of DynaBeads (DynaL A.S., Oslo, Norway). After washing, bound mRNA was eluted into reverse transcription buffer (Gibco BRL, Gaithersburg, Md). Ten μ g of mRNA was used to generate cDNA through use of Superscript II (Gibco BRL). PCR primers, 5'-ATGAGGATGCTTCTGCATTGAGTTTGCTA-3' and 5'TCTAGATTAACCTTTCTATTATCCA-3', specific to human IL-5, and a PCR program of 30 cycles of 94°C for 1 minute, 52°C for 2 minutes, and 72°C for 1.5 minutes were used to amplify the monkey IL-5 gene. PCR product was gel-purified and ligated into pCR2.1 vector (Invitrogen, Carlsbad, Calif). DNA of 3 independent monkey IL-5 clones had identical sequences.

TABLE I. Effect of rhIL-5 and recombinant monkey IL-5 on eosinophil differentiation and proliferation of IL-5 receptor-positive cells and neutralization activity of mepolizumab

	Eosinophil differentiation in bone marrow		TF-1 cell proliferation	B13 cell proliferation
	Human	Monkey		
IL-5 EC ₅₀ (pM)				
Human IL-5	13.3	13.3	0.7	2.7
Monkey IL-5	13.3	13.3	0.7	6.0
Mepolizumab IC ₅₀ (pM)				
Human IL-5	70	104	75	83
Monkey IL-5	83	116	84	79

EC₅₀, Effective concentration required for 50% inhibition; IC₅₀, Concentration required for 50% inhibition.

Monkey IL-5 was expressed in stably transfected *Drosophila* S2 cells, as described previously for human IL-5,¹⁷ with the following modifications. The monkey IL-5 coding region was amplified from cDNA using forward primer 5'-TCCCCGCGGGCGGCCATGAGGATGCTTCTGCATTTG (*Sac*I site and initiator methionine codon underlined) and reverse primer 5'-GCTCTAGATTAAGTTTCTATATCCACTC (*Xba*I site underlined). PCR product was digested with *Sac*I and *Xba*I and subcloned into corresponding restriction sites of the expression vector used for rhIL-5.

Biologic activity of recombinant monkey IL-5. Recombinant human and monkey IL-5-induced proliferation was assessed on the human erythroleukemic cell line TF-1 (ATCC; subclone TF-1.28, prepared by SmithKline Beecham). This subclone expresses functional IL-5R (α and β chains). Proliferation was also assessed on the mouse pre-B cell line B13 (obtained courtesy of R. Palacios, Basel Institute of Immunology, Basel, Switzerland). In our hands, B13 cells respond to human and murine IL-5. Assays were conducted in microtiter plates containing cells, IL-5 (7 or 35 pM), and mepolizumab. Proliferation was measured by ³H-thymidine incorporation over the last 4 hours of culture.

Human or monkey bone marrow mononuclear cells were incubated with rhIL-5 or monkey IL-5 (10 ng/mL) and increasing concentrations of mepolizumab over 14 to 21 days. Eosinophil-peroxidase-positive cells were determined spectrophotometrically at the start of culture and at intervals thereafter.

Leukocyte phenotyping. Femoral blood samples were analyzed for lymphocyte markers (CD2, CD4, CD8, CD16, and CD20) or granulocyte markers (CD11b and CD49d; Becton Dickinson, San Diego, Calif). To measure eosinophil activation, the flow cytometer was gated to display eosinophils on the basis of forward and side scatter and CD49d staining.¹⁸ The intensity of CD11b staining was recorded as a measure of eosinophil activation.¹⁹

Peripheral blood mitogen responses. Femoral blood samples were collected into heparinized tubes for analysis of mitogen responses (PHA, PWM, and Con A), as described previously.²⁰

Bone marrow analysis. Bone marrow eosinophils were assessed in histologic sections of rib collected at termination of the 6-month study and stained by Luna's method.²¹ For each slide, 10 high-power fields (HPF; 500 $\times$) were evaluated through use of an eyepiece reticle (1 cm $\times$ 1 cm). Fields were chosen on the basis of cellularity and staining uniformity. The eosinophils in 10 HPFs were counted and classified as mature (segmented) or immature (blast-to-band forms).

Pharmacokinetics. Heparinized femoral blood samples were analyzed for mepolizumab through use of an electrochemiluminescent immunoassay with a dynamic range of 50 to 5000 ng/mL, as described previously.²²

Antigenicity of mepolizumab. Serum samples (diluted 1:5 in PBS/0.1% BSA) were incubated with TAG (ruthenium)-mepolizumab and biotin-mepolizumab for 1 hour; this was followed by incubation, without washing, with streptavidin-coated paramagnetic microbeads for 30 minutes. ORIGIN assay buffer was added and electrochemiluminescent responses recorded on an ORIGIN Analyzer (IGEN International, Gaithersburg, Md). The assay dynamic range was 94 to 6000 ng/mL.

Bronchoalveolar lavage. BAL samples were collected before initiation of drug treatment, on day 104 of the 6-month study, and at listed times in the pharmacology study. Each monkey was anesthetized by means of an intramuscular injection of ketamine with or without rompum (10 and 1 mg/kg, respectively). A topical anesthetic was applied to the larynx and a pediatric bronchoscope inserted down into a lung lobe until resistance was detected. Warmed saline solution (37°C) was instilled and withdrawn in two to three 13- to 20-mL aliquots. Lavage fluid was pooled and centrifuged, and supernatants were removed. Cell pellets were resuspended in 2 mL of PBS and aliquots processed for total and differential leukocyte enumeration. Supernatants were concentrated 5-fold by ultrafiltration (10-kD filter) or dried under nitrogen gas and resuspended in a minimal volume. Lavage fluid was analyzed for total protein, total IgG, and mepolizumab in the 6-month study and for the cytokines RANTES, IL-6, and IL-8 (human ELISA kits, R&D Systems, Minneapolis, Minn) in the pharmacology study.

Statistical analysis

Results were analyzed by means of 1-way ANOVA or Student *t* testing. A *P*-value of less than .05 was interpreted as the level of statistical significance.

RESULTS

Initial evaluation of mepolizumab activity against IL-5 from various animal species used for pharmacology and toxicology studies (mouse, rat, guinea pig, dog, and monkey) found that mepolizumab would recognize and neutralize only IL-5 from primates. To compare mepolizumab's activity against human IL-5 and its activity against monkey IL-5, cynomolgus monkey (*Macaca fascicularis*) IL-5 was cloned and expressed. The amino acid sequence of monkey IL-5 differs from human IL-5 by 2 conservative substitutions (Ala27Gly and Asn40His). Interestingly, the reported sequence of rhesus monkey (*Macaca mulata*) IL-5 also differs from human IL-5 by 2 amino acids, but in different locations

(Thr4Ala and Asp77Gly).²³ Importantly, amino acids critical for IL-5 functions—ie, C terminus residues, critical for binding, and N terminus Glu11, critical for signaling,^{24,25,26}—are conserved.

Recombinant monkey IL-5 and rhIL-5 were equipotent in *in vitro* proliferative responses for IL-5 receptor-positive cell lines and inducing eosinophil differentiation in bone marrow cultures (Table I). Mepolizumab equally inhibited the biological activity of rhIL-5 and recombinant monkey IL-5 in all assays. We therefore concluded that cynomolgus monkeys were an appropriate species in which to conduct pharmacology and toxicity studies for mepolizumab.

Single-dose pharmacology study

Two weeks before drug treatment, mean BAL eosinophil counts increased 6- to 10-fold 24 hours after challenge with aerosolized *A suum* (Fig 1). One day after drug administration, BAL eosinophils increased 2-fold for both groups after challenge; however, mepolizumab-treated monkeys had 60% fewer eosinophils. At 3 weeks, there was a minimal increase in BAL eosinophils in mepolizumab-treated monkeys ($P < .05$); in the vehicle-treated group, there was a 5-fold increase. Although the difference was not statistically significant at 6 weeks, BAL eosinophils in the vehicle-treated monkeys increased 5-fold whereas they were only mildly increased in mepolizumab-treated monkeys. Increases in eosinophils were accompanied by comparable increases in total BAL cell counts, indicating that eosinophils were the primary cell type infiltrating into the lungs.

Peripheral blood eosinophil counts were reduced approximately 50% in comparison with prestudy values in mepolizumab-treated monkeys by 48 hours after dosing; this reduction lasted through 6 weeks (Fig 1). All other hematologic parameters were unaffected.

There was no effect of mepolizumab on acute bronchoconstrictor response to antigen immediately after challenge at any of the timepoints evaluated, as assessed by R_L and C_{DN} (data not shown). This model has not been validated to assess late-phase responses.

BAL samples collected before and after exposure to *A suum* at 3 weeks after dosing were analyzed by means of ELISA for cytokines found to be elevated in asthmatic patients.²⁷ There was significantly less RANTES ($P < .025$) and a trend toward reduced IL-6 ($P = .061$) in BAL from monkeys that received mepolizumab; in contrast, there was no effect on IL-8 levels (Table II).

Single-dose safety pharmacology and toxicity studies

Single doses up to 100 mg/kg had no effect on body temperature or on cardiovascular, respiratory, and renal function in male monkeys. A single dose of 300 mg/kg was well tolerated by monkeys over a 1-month observation period with no effects on immune function.

Two-dose toxicity and pharmacology study

Mepolizumab induced a dose-dependent decrease in peripheral blood eosinophils that reached a nadir 1

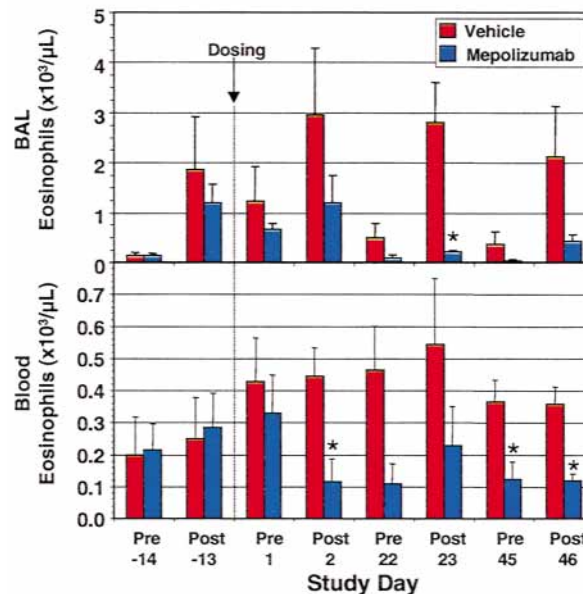


FIG 1. Eosinophils in BAL fluid and blood of *A suum*-sensitive monkeys. Samples were collected before (*Pre*) *A suum* challenge and 24 hours later (*Post*). Mepolizumab reduced circulating blood eosinophils and blocked the increase in circulating and BAL eosinophils after challenge. * $P < .05$ versus vehicle.

month after dosing. A maximal decrease ($P < .05$) of up to 94% was observed at doses of 5 mg/kg or greater (Fig 2). This decrease was maintained through day 77 ($P < .05$ for doses of 5 mg/kg). Eosinophil counts returned to prestudy values by day 99.

Administration of rhIL-2 led to a maximal increase in circulating eosinophils on day 41 (24 hours after the sixth dose), when mean eosinophil counts were increased to $5.54 \times 10^9/L$ (range, 0.99 – $11.83 \times 10^9/L$) in comparison with a mean baseline value of $0.11 \times 10^9/L$ (range, 0.02 – $0.19 \times 10^9/L$; Fig 2). This increase was significantly blocked ($P < .05$) by $\geq 95\%$ in monkeys given 5 or 50 mg/kg mepolizumab and 85% to 97% blunted ($P < .05$) in monkeys given 0.5 mg/kg mepolizumab. Mepolizumab and rhIL-2 did not affect eosinophil activation.

Six-month toxicity study

All doses were well tolerated with no evidence of clinical or immunologic effects. There were no drug-related histopathologic findings, including sc injection sites. A significant decrease ($P < .05$) in circulating eosinophils (80% to 99%) was observed in all mepolizumab-dosed monkeys (Fig 3, *top*). Analysis of bone marrow eosinophils at study termination found no significant effect of mepolizumab on immature or mature eosinophils (Table III). Histopathologic examination of tissues known to contain elevated levels of eosinophils (eg, lung and small intestine) did not note differences in eosinophil distribution or apparent numbers of tissue eosinophils. No effects were seen on leukocyte subsets (data not shown).

In BAL samples, eosinophils were reduced on day 104 in comparison with prestudy values ($P < .05$ for 10 mg/kg sc

TABLE II. BAL cytokines before and 24 hours after challenge with *A suum* on day 22

	RANTES		IL-6		IL-8	
	Baseline	24 h	Baseline	24 h	Baseline	24 h
Vehicle	14.40 ± 4.23	34.06 ± 7.45	2.84 ± 0.46	10.61 ± 3.17	4.27 ± 4.13	33.63 ± 20.66
Mepolizumab	15.56 ± 4.60	12.78 ± 6.63*	3.30 ± 1.51	5.01 ± 2.39	7.04 ± 7.04	38.88 ± 31.03

Each value is mean ± SEM.

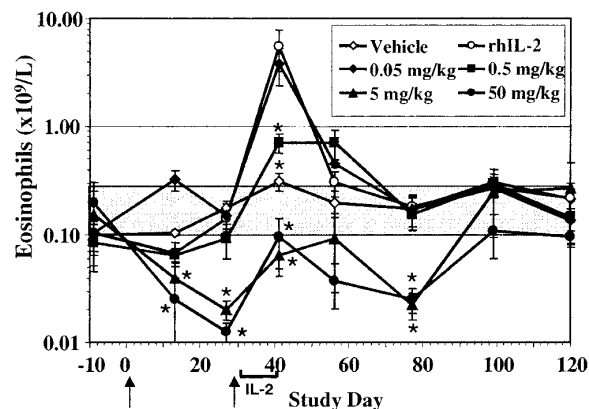
* $P < .05$.

FIG 2. Peripheral blood eosinophil counts from the pharmacology and toxicity study. Monkeys received intravenous doses of mepolizumab (arrows) and 6 subcutaneous doses of rhIL-2 (bracket). Shaded region marks the 25th and 75th percentiles of control eosinophil counts over the course of the study. * $P < .05$ versus vehicle.

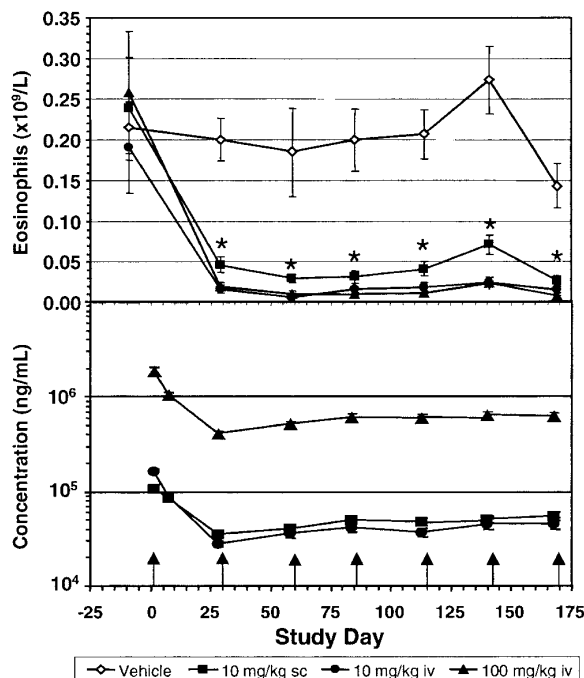


FIG 3. Top panel, Peripheral blood eosinophil counts from monkeys in the 6-month toxicity study. Bottom panel, Plasma concentration versus time profiles from monkeys dosed for 6 months with mepolizumab. Arrows indicate dosing days. The clearance profile after the first dose is illustrated; the remaining time points are trough sampling time points. * $P < .05$ versus vehicle.

and 100 mg/kg iv; $P = .052$ for 10 mg/kg iv) in all mepolizumab-treated monkeys (Fig 4, top). BAL fluid contained dose-proportional concentrations of mepolizumab that were approximately 500-fold less than in plasma (Fig 4, middle). Normalization of mepolizumab to IgG values in blood and BAL showed a strong correlation ($P < .001$) of mepolizumab-to-IgG concentrations in both compartments, suggesting passive transfer of mepolizumab along with IgG from vascular to alveolar compartments (Fig 4, bottom).²⁸

Pharmacokinetics

After a single iv dose of 300 mg/kg, mean maximal plasma concentrations (C_{max}) up to 7.0 mg/mL and $AUC_{(0-inf)}$ up to 1450 mg · hr/mL were observed. In single- and 2-dose studies, the terminal half-life of mepolizumab was determined to be 13 ± 2 days. In the 6-month study, trough plasma concentrations (samples collected just before each monthly dose) plateaued after the fourth monthly dose (Fig 3, bottom). AUC values calculated after iv and sc dosing were comparable, indicating that mepolizumab was well absorbed from sc injection sites. Exposure was approximately dose-proportional over a range of 0.05 to 300 mg/kg, and there were no marked exposure differences between male and female monkeys.²²

Antigenicity of mepolizumab.

Assessment of sera from single- and repeat-dose iv and sc toxicity studies for monkey antimepolizumab antibodies did not indicate any positive responses.

DISCUSSION

In studies of mepolizumab in monkeys, in vivo pharmacologic activity leading to decreases in eosinophil counts was observed. In a 2-dose study, doses at or greater than 5 mg/kg produced a decrease of up to 85% in basal peripheral blood eosinophil counts as early as 8 days after the first dose. Counts remained >85% below control values for at least 11 weeks in monkeys that received 5 mg/kg or greater. Interestingly, after a second administration, doses at or greater than 0.5 mg/kg blocked rhIL-2-induced eosinophilia, whereas this dose had no effect on basal eosinophil counts. This suggests that the sites of production of IL-5 for these 2 processes are different—ie, lymphocytes in blood or lymphatic tissues for IL-2-driven eosinophilia²⁹ and bone marrow for basal eosinophil production—and that the distribution of mepolizumab into these respective compartments is dose-dependent. Thus, lower circulating levels of mepolizumab would be needed to block IL-5 produced at sites of allergic disease, such as the lung. In the 6-month toxicity study, peripher-

al blood eosinophil counts were decreased 80% to 99% for the study duration with no adverse effects. The persistence of low numbers of eosinophils in mepolizumab-treated monkeys reflects the complex interrelationships and redundancies in the immune system, where GM-CSF and IL-3 also play a role in eosinophil production.³⁰ Indeed, in transgenic mice in which the gene for IL-5 has been disrupted, circulating eosinophils are present, but at levels below those in normal mice.³¹

Analysis of eosinophil lineages in bone marrow from the 6-month study found no significant effect of mepolizumab on immature or mature eosinophils. Thus there appears to be a blockade of progenitor cells entering into early eosinophil maturation in the bone marrow by treatment with anti-IL5 antibody, and not a depletion or accumulation of eosinophil lineage cells. Accordingly, in contrast to other antibody therapies that reduce or deplete immune cell numbers, such as anti-CD4 mAbs,³² recovery of circulating eosinophils following discontinuation of mepolizumab treatment is complete after systemic clearance of the antibody. Suppression of eosinophilic responses to allergens has been observed in mice that were given TRFK-5; there was a similar recovery in eosinophil numbers after mAb clearance from circulation.³³

Mepolizumab clearance, which is comparable with endogenous IgG, is beneficial for treatment of chronic diseases, such as asthma, in which monthly or quarterly dosing is feasible. However, because of slow clearance and prolonged recovery of eosinophils, there is a risk of reduced immune responses. The requirement for IL-5 in host defense against parasitic infection, a disease in which an eosinophilic response is believed beneficial, is questionable, inasmuch as there was no increase in worm burden or worsening of pathosis in IL-5 knockout mice infected with *Mesocostoides corti*.³¹ In the present studies, there was no evidence of immunosuppression in monkeys that had markedly reduced eosinophil counts for more than 6 months.

Eosinophilia in response to administration of rhIL-2 occurs in numerous species, including mice, rats, cats, dogs, monkeys, and human beings, by stimulating CD4-positive T cells to produce and secrete IL-5.²⁹ Increased levels of IL-5 were detected in patients undergoing rhIL-2 therapy,³⁴ and rhIL-2-induced eosinophilia in mice can be suppressed with an anti-IL-5 antibody.³⁵ rhIL-2 was well tolerated by monkeys and produced a profound eosinophilia—as high as 50% of white blood cells. In monkeys receiving mepolizumab, there was a dose-dependent inhibition of rhIL-2-induced eosinophilia, with doses of 0.5 mg/kg or greater producing a >80% inhibition in this model. Interestingly, a TRFK-5 dose of 0.3 mg/kg reduced eosinophil recruitment into airways of *A suum*-sensitive cynomolgus monkeys.¹¹

A pharmacokinetic/pharmacodynamic analysis of mepolizumab plasma concentrations versus eosinophil counts was previously reported for monkeys in which a concentration required for 50% inhibition (IC₅₀) of 1.4 µg/mL for reduction of eosinophil counts was estimated for steady-state reduction.²² We observed a delay in

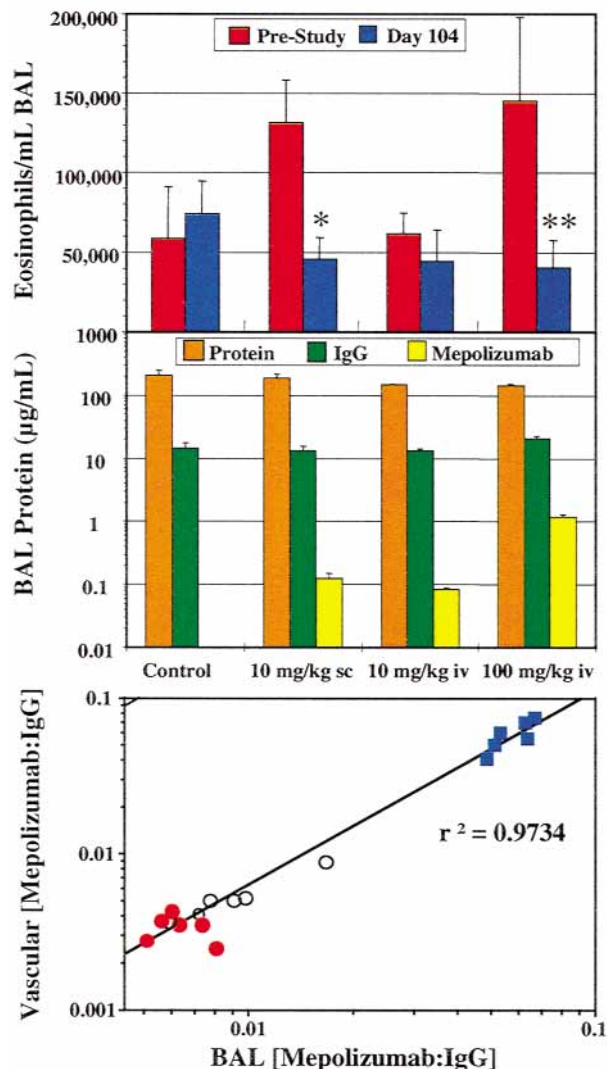


FIG 4. BAL results from the 6-month toxicity study. *Top panel* shows that the number of eosinophils recovered in BAL decreases in monkeys given mepolizumab (* $P < .05$; ** $P = .052$); this compares with a slight increase in vehicle-treated monkeys. *Middle panel* presents quantitation of total protein, IgG, and mepolizumab in BAL fluid. *Bottom panel* plots the ratios of mepolizumab-to-IgG in blood and BAL fluid. ○, 10 mg/kg iv-dosed monkeys; ■, 100 mg/kg sc-dosed monkeys.

TABLE III. Effect of 6 months' mepolizumab treatment on bone marrow eosinophils

Treatment*	Immature†	Mature (segmented)†
Control (vehicle)	8.1 ± 3.3	40.3 ± 17.5
10 mg/kg (sc)	5.4 ± 1.0	33.2 ± 12.2
10 mg/kg (iv)	3.6 ± 1.1	23.0 ± 9.5
100 mg/kg (iv)	3.7 ± 3.1	27.3 ± 15.5

sc, Subcutaneous injection. iv, intravenous administration.

*n = 6/group (3 males, 3 females).

†Number of cells per 10 HPFs ± SEM.

reaching the eosinophil nadir of 3 to 4 weeks after dosing. This is an interesting observation, because during the first month after dosing, plasma concentrations of

mepolizumab are high; it is most likely due to the fact that mepolizumab affects eosinophils indirectly by neutralizing IL-5.³⁶ On study day 120 in monkeys dosed with 50 mg/kg mepolizumab, mean plasma concentrations of approximately 11 µg/mL did not affect circulating eosinophil counts; in contrast, mean plasma concentrations of 5 µg/mL in the 5 mg/kg group were associated with decreased eosinophil counts on study day 77. This loss of effect on reducing circulating eosinophil counts at low plasma concentrations could be due to compensating mechanisms in the bone marrow—ie, IL-3 or GM-CSF—that play a role in eosinophil production.³⁰

Mepolizumab was evaluated in an acute model of asthma in cynomolgus monkeys, which have strong bronchoconstrictor response to *A suum* antigen. Mepolizumab did not attenuate the acute bronchoconstrictor response to antigen challenge. A definitive role for IL-5 and eosinophils in murine models of allergic airway disease³⁷⁻⁴¹ is apparently dependent on mouse strain and sensitization protocols used.⁴² The present study results are consistent with those reported for murine ovalbumin models^{37,41} and for clinical data in antigen-challenged asthmatic patients.¹⁴

Mepolizumab treatment led to a marked inhibition of pulmonary and peripheral blood eosinophilia in response to *A suum* challenge; this lasted for at least 6 weeks and was similar to the long-term effects for anti-IL-5 mAbs in monkeys.⁴³ In addition, reduction in BAL concentrations of RANTES and IL-6 was detected. Elevated levels of these cytokines in patients with asthma²⁷ are believed to play a role in pathologic remodeling of lung airways. By affecting both numbers of eosinophils in lungs and levels of proinflammatory cytokines, mepolizumab might affect the long-term remodeling of airways that is characteristic of asthma.⁴⁴

Antigenicity of monoclonal antibodies in clinical use has been dramatically reduced since the shift from murine to humanized mAbs.⁴⁵ However, even in preclinical studies with humanized antibodies in monkeys whose immunoglobulins are >90% homologous to man,⁴⁶ antibody responses are reported. The results of antibody responses in monkeys varies from enhanced mAb clearance from circulation⁴⁷ to anaphylactoid reactions (unpublished data). In studies reported here, neither repeated iv administration nor sc administration of mepolizumab induced antibody responses.

In conclusion, the potential toxicity of mepolizumab administration was evaluated in single- and repeat-dose studies in monkeys. Mepolizumab was well tolerated, with no evidence of systemic or local (injection site) reactivity. When a rollover study design was used, monkeys were exposed to intermittent dosing with no adverse or immunologic effects. These safety and efficacy results support chronic or intermittent use of mepolizumab for diseases in which eosinophils are a predominant cell type, such as asthma, or for seasonal disorders, such as allergic rhinitis. Chronic therapy with this antibody will most likely be required, because the short-term reduction in eosinophils, as shown in these studies and recent clinical trials,^{14,15} does not affect pulmonary function endpoints.

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REFERENCES

1. Drazen J, Arm JP, Austen FK. Sorting out the cytokines of asthma. *J Exp Med* 1996;183:1-5.
2. Busse WW, Calhoun WF, Sedgewick JD. Mechanism of airway inflammation in asthma. *Am Rev Res Dis* 1993;147:S20-S24.
3. Reed CE. The importance of eosinophils in the immunology of asthma and allergic disease. *Ann Allergy* 1994;72:376-80.
4. Sanderson CJ. Interleukin-5: an eosinophil growth and activation factor. *Dev Biol Standard* 1988;69:23-9.
5. Clutterbuck EJ, Hirts EME, Sanderson CJ. Human interleukin-5 (IL-5) regulated production of eosinophils in human bone marrow cultures: comparison and interaction with IL-1, IL-3, IL-6 and GM-CSF. *Blood* 1989;73:1504-13.
6. Wang JM, Rimbaldi A, Biondi A, Chen ZG, Sanderson CJ, Mantovani A. Recombinant IL-5 is a selective eosinophil chemoattractant. *Eur J Immunol* 1989;19:701-5.
7. Lopez AF, Sanderson CJ, Gamble JR, Campbell HD, Young IG, Vadas MA. Recombinant human interleukin-5 is a selective activator of human eosinophil function. *J Exp Med* 1988;167:219-24.
8. Hamelmann E, Gelfand EW. Role of IL-5 in the development of allergen-induced airway hyperresponsiveness. *Int Arch Allergy Immunol* 1999;120:8-16.
9. Lalani T, Simmons RK, Ahmed AR. Biology of IL-5 in health and disease. *Ann Allergy Asthma Immunol* 1999;82:317-33.
10. Van Oosterhout AJ, Fattah D, Van Ark I, Hofman G, Buckley TL, Nijkamp FP. Eosinophil infiltration precedes development of airway hyperreactivity and mucosal exudation after intranasal administration of interleukin-5 to mice. *J Allergy Clin Immunol* 1995;96:104-12.
11. Mauser PJ, Pitman AM, Fernandez X, Foran SK, Adams GK, Kruetner W, et al. Effects of an antibody to interleukin-5 in a monkey model of asthma. *Am J Respir Crit Care* 1995;152:467-72.
12. Akutsu I, Kojima T, Kariyone A, Fukuda T, Makino S, Takatsu K. Antibody to interleukin-5 prevents antigen-induced eosinophil infiltration and bronchial hyperreactivity in the guinea pig airway. *Immunol Lett* 1995;45:109-16.
13. Underwood DC, Osborne RR, Newsholme SJ, Torphy TJ, Hay DW. Persistent airway eosinophilia after leukotriene (LT) D₄ by the LTD₄ receptor antagonist pranlukast, or an interleukin-5 monoclonal antibody. *Am J Respir Crit Care* 1996;154:850-7.
14. Leckie MJ, Brinke AT, Khan J, Diamant Z, O'Connor J, Walls CM, et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 2000;356:2144-2148.
15. Bryan SA, O'Connor BJ, Matti S, Leckie MJ, Kanabar V, Khan J, et al. Effects of recombinant human interleukin-12 on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 2000;356:2149-53.
16. Ames R, Tornetta M, McMillan L, Kaiser K, Holmes S, Appelbaum E, et al. Comparison of neutralizing murine monoclonal antibodies to human interleukin-5 isolated from hybridomas and a filamentous phage Fab display library. *J Immunol* 1995;154:6355-6364.
17. Johanson K, Appelbaum E, Doyle M, Hensley P, Zhao B, Abdel-Meguid SS, et al. Binding interactions of human interleukin 5 with its receptor alpha subunit. Large scale production, structural, and functional studies of *Drosophila*-expressed recombinant proteins. *J Biol Chem* 1995;270:9459-71.
18. Thureau AM, Schlyz U, Wolf V, Schauer U. Identification of eosinophils by flow cytometry. *Cytometry* 1996;23:150-158.
19. Rodgers S, Rees RC, Hancock BW. Changes in the phenotypic characteristics of eosinophils from patients receiving recombinant human interleukin-2 (rhIL-2) therapy. *Br J Haematol* 1994;86:746-53.
20. Bugelski PJ, Thiem PA, Trunch A, Morgan DG. Recombinant human soluble CD4 does not inhibit immune function in cynomolgus monkeys. *Toxicol Pathol* 1991;19:580-588.

21. Prophet EB, Mills B, Arrington JA, Sobin LH, editors. Laboratory methods in histotechnology. Washington DC: American Registry of Pathology; 1994. p. 146.
22. Zia-Amirhosseini P, Minthorn E, Benincosa LJ, Hart TK, Hottenstein CS, Tobia LAP, Davis CB. Pharmacokinetics and pharmacodynamics of SB-240563, a humanized monoclonal antibody directed to human interleukin-5, in monkeys. *J Pharm Exp Ther* 1999;291:1060-7.
23. Villinger F, Brar SS, Mayne A, Chikkala N, Ansari AA. Comparative sequence analysis of cytokine genes from human and nonhuman primates. *J Immunol* 1995;155:3946-54.
24. Morton T, Li J, Cook R, Chaikin I. Mutagenesis in the C-terminal region of human interleukin 5 reveals a central patch for receptor α chain recognition. *Proc Natl Acad Sci U S A* 1995;92:10879-10883.
25. Tavernier J, Tuypens T, Verhee A, Platinck G, Devos R, van der Heyden J, et al. Identification of receptor-binding domains on human interleukin 5 and design of an interleukin 5-derived receptor antagonist. *Proc Natl Acad Sci U S A* 1995;92:5194-8.
26. Graber P, Proudfoot AEI, Talabot F, Bernard A, McKinnon M, Banks M, et al. Identification of key charged residues of human interleukin-5 in receptor binding and cellular activation. *J Biol Chem* 1995;26:15762-9.
27. Konno S-I, Gonokami Y, Kurokawa M, Kawazu K, Asano K, Okamoto K-I, et al. Cytokine concentrations in sputum of asthmatic patients. *Int Arch Allergy Immunol* 1996;109:73-8.
28. Out TA, Van de Graaf EA, Van den Berg NJ, Jansen HM. IgG subclasses in bronchoalveolar lavage fluid from patients with asthma. *Scand J Immunol* 1991;33:719-27.
29. Macdonald D, Gordon AA, Kahitani H, Enokihara H, Barrett AJ. Interleukin-2 treatment-associated eosinophilia is mediated by interleukin-5 production. *Br J Haematol* 1990;76:168-173.
30. Tai PC, Sun L, Spry CJ. Effects of IL-5, granulocyte/macrophage colony-stimulating factor (GM-CSF) and IL-3 on the survival of human blood eosinophils in vitro. *Clin Exp Immunol* 1991;85:312-6.
31. Kopf M, Brombacher F, Hodgkin PD, Ramsay AJ, Milbourne EA, Dai WJ, et al. IL-5-deficient mice have a developmental defect in CD5+ B-1 cells and lack eosinophilia but have normal antibody and cytotoxic T cell responses. *Immunity* 1996;4:15-24.
32. Moreland LW, Pratt PW, Bucy RP, Jackson BS, Feldman JW, Koopman WJ. Treatment of refractory rheumatoid arthritis with a chimeric anti-CD4 monoclonal antibody: long-term follow-up of CD4+ T cell counts. *Arthritis Rheum* 1994;37:834-8.
33. Garlisi CG, Kung TT, Wang P, Minnicozzi M, Umland SP, Chapman RW, et al. Effects of chronic anti-interleukin-5 monoclonal antibody treatment in a murine model of pulmonary inflammation. *Am J Resp Cell Mol Biol* 1999;20:248-55.
34. Pisani CH, Kovach JS, Kita H, Keiferman KM, Gleich GJ, Silver JE, et al. Administration of interleukin-2 (IL-2) results in increased plasma concentrations of IL-5 and eosinophilia in patients with cancer. *Blood* 1991;78:1538-44.
35. Yamaguchi Y, Suda T, Shiozaki J, Miura Y, Hitoshi Y, Tominaga A, et al. Role of IL-5 in IL-2-induced eosinophilia. In vivo and in vitro expression of IL-5 mRNA by IL-2. *J Immunol* 1990;145:873-7.
36. Shi H-Z, Humbles A, Gerard C, Jin X, Weller PF. Lymph node trafficking and antigen presentation by endobronchial eosinophils. *J Clin Invest* 2000;105:945-53.
37. Corry DB, Grunig G, Hadeiba H, Kurup VP, Warnock ML, Sheppard D, et al. Requirements for allergen-induced airway hyperactivity in T and B cell-deficient mice. *Mol Med* 1998;4:344-55.
38. Mould AW, Ramsay AJ, Matthaie KI, Young IG, Rothenberg ME, Foster PS. The effect of IL-5 and eotaxin expression in the lung on eosinophil trafficking and degranulation and the induction of bronchial hyperactivity. *J Immunol* 2000;164:2142-50.
39. Lee JJ, McGarry MP, Farmer SC, Denzler KL, Larson KA, Carrigan PE, et al. Interleukin-5 expression in the lung epithelium of transgenic mice leads to pulmonary changes pathognomonic of asthma. *J Exp Med* 1997;185:2143-56.
40. Hogan SP, Koskinen A, Foster PS. Interleukin-5 and eosinophils induce airway damage and bronchial hyperactivity during allergic airway inflammation in BALB/c mice. *Immunol Cell Biol* 1997;75:284-8.
41. Corry DB, Folkesson HG, Warnock ML, Erle DJ, Matthay MA, Wiener-Kronish JP, et al. Interleukin 4, but not interleukin 5 or eosinophils, is required in a murine model of acute airway hyperactivity. *J Exp Med* 1996;183:109-17.
42. Brewer JP, Kisselgof AB, Martin TR. Genetic variability in pulmonary responses to antigen in mice. *Am J Resp Crit Care Med* 1999;160:1150-6.
43. Egan RW, Diljeet A, Bodmer MW, Carter JM, Chapman RW, Chou C-C, et al. Effect of Sch 55700, a humanized monoclonal antibody to human interleukin-5, on eosinophilic responses and bronchial hyperactivity. *Drug Res* 1999;49:779-90.
44. Holgate ST, Lackie PM, Davies DE, Roche WR, Walls AF. The bronchial epithelium as a key regulator of airway inflammation and remodelling in asthma. *Clin Exp Allergy* 1999;29 Suppl 2:90-5.
45. Vaswani SK, Hamilton RG. Humanized antibodies as potential therapeutic drugs. *Ann Allergy Asthma Immunol* 1998;81:105-19.
46. Newman R, Alberts J, Anderson D, Carner K, Heard C, Norton R, et al. "Primatization" of recombinant antibodies for immunotherapy of human diseases: a macaque/human chimeric antibody against human CD4. *Biotechnology* 1992;10:1455-60.
47. Hakimi J, Chizzonite R, Luke DR, Familletti PC, Bailon P, Kondas JA, et al. Reduced immunogenicity and improved pharmacokinetics of humanized anti-Tac in cynomolgus monkeys. *J Immunol* 1991;147:1352-9.